UNIVERSITI PUTRA MALAYSIA

IN VITRO VIABILITY AND ULTRASTRUCTURAL CHANGES OF CRYOPRESERVED IMMATURE BOVINE OOCYTES

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By

MYINT THEIN

Thesis Submitted to the School of Graduate Studies
Universiti Putra Malaysia in Fulfilment of the Rrequirements for the
Degree of Doctor of Philosophy

January 2003

UPM

DEDICATION

This thesis is dedicated

To My Teachers and My Parents

For their profound gratitude

And

To My Wife, Daughter and Son

For their eternal love

Abstract of dissertation submitted to the Senate of Universiti Putra Malaysia
in fulfilment of the requirements for the degree of Doctor of Philosophy

IN VITRO VIABILITY AND ULTRASTRUCTURAL CHANGES OF CRYOPRESERVED IMMATURE BOVINE OOCYTES

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January 2003

Chairman : Assoc. Prof. Dr. Abd. Wahid Haron, D.V.M., Ph.D.

Faculty : Veterinary Medicine

Several studies have shown that current cryopreservation procedures are severely detrimental to the viability of immature bovine oocytes and permit fertilization and development at a very reduced rate. In this study, a number of experiments were conducted to determine the *in vitro* viability of frozen-thawed and vitrified-thawed immature bovine oocytes.

In vitro viability of frozen-thawed immature bovine oocytes was determined based on cumulus mass expansion, nuclear maturation, cleavage and blastocyst rates. Viability was assessed following experiments conducted using a variety of cooling starting temperatures, seeding temperatures, permeable cryoprotectants and saccharides. Effect of using follicular fluid in the preparation of freezing solution on the viability of immature bovine oocytes was also examined. During freezing, chilling injury and

cryoprotective agents impaired the viability of immature oocytes. Among the initial cooling temperatures tested, 30°C yielded the best maturation (34.4%) and cleavage (4.5%) rates and while maturation, cleavage and blastocyst rates from unfrozen oocytes were 86.7%, 69.5% and 17.4%, respectively. As for the permeable cryoprotectants, ethylene glycol was the least toxic compared to propanediol and dimethyl sulphoxide. In the experiment of viability study of oocytes after exposure to freezing solution, significantly better cleavage and blastocyst rates were observed when follicular fluid from >15-mm follicles was added in freezing solution. However, maturation and cleavage rates following freezing with follicular fluid were statistically significant. Follicular fluid may have the beneficial effect by protecting oocytes from the toxicity of freezing solution but it may not have enough protective property against freezing *per se*.

The maturation rate of immature oocytes was severely affected when exposed to vitrification solution (39.6%) and vitrifying-thawing procedure (33.9%). However, maturation rate of vitrification solution-exposed oocytes did not differ significantly from that of vitrified-thawed oocytes. These results indicate that the adverse effect on maturation rate is mainly due to vitrification solution rather than vitrification procedure.

Any ultrastuctural alterations resulted from freezing and vitrification procedures were investigated using the transmission electron microscopy in order to facilitate a better understanding of the cause of the low viability. Enlarged perivitelline space and fewer microvilli were common ultrastructural alterations that resulted from cryopreservation.

Despite impairment on the viability of oocytes, most organelles of cryopreserved oocytes were able to retain their morphology.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

KEUPAYAAN HIDUP *IN VITRO* DAN PERUBAHAN ULTRASTRUKTUR OOSIT BOVIN TIDAK MATANG YANG DISEJUKBEKUKAN

Oleh

MYINT THEIN
Januari 2003

Pengerusi : Prof Madya Dr. Abd. Wahid Haron, D.V.M., Ph.D.

Fakulti : Perubatan Veterinar

Beberapa kajian menunjukkan prosedur penyejukbekuan terkini yang dilakukan ke atas oosit bovin tidak matang mengakibatkan kegagalan keupayaan hidup yang teruk dan pengurangan kadar persenyawaan dan perkembangannya. Dalam kajian ini, beberapa ujian dilakukan untuk mengenal pasti keupayaan hidup secara *in vitro* oosit bovin tidak matang yang disejukbeku dan divitrifikasi.

Keupayaan hidup in vitro oosit tidak matang bovin ditentukan berdasar pengembangan jisim kumulus, pematangan nukleus, kadar pembelahan dan blastosis. Keupayaan hidup dinilai ujikaji dikendalikan secara penentuan suhu permulaan penyejukbekuan, suhu seeding, sakarid dan bahan penebat sejuk boleh serap. Kesan penggunaan cecair folikel dalam penyediaan larutan penyejukbekuan ke atas keupayaan hidup oosit tidak matang bovin juga diperiksa. Penyejukbekuan mengurangkan keupayaan hidup oosit tidak

matang. Di kalangan suhu permulaan penyejukbekuan yang diuji, 30°C memberikan kadar pematangan (34.4%) dan pembelahan (4.5%) yang terbaik sementara kadar pematangan. pembelah dan blastosis bagi oosit yang tidak disejukbeku masing-masing adalah 86.7%, 69.5% dan 17.4%. Bagi larutan penyejukbekuan mudah resap, etilene glikol didapati sangat kurang toksik berbanding propanediol dan dimetil sulfoksida. Dalam ujian keupayaan hidup oosit selepas terdedah kepada larutan pembekuan, kadar pembelahan dan blastosis yang lebih bererti diperolehi apabila cecair folikel bersaiz >15mm dicampurkan dalam larutan pembekuan. Walau bagaimanapun, tiada perbezaan dalam kadar pematangan dan pembelahan diperolehi selepas disejukbeku dengan cecair folikel. Cecair folikel berkemungkinan mempunyai kesan baik untuk melindungi oosit daripada kesan toksik larutan pembekuan tetapi tidak mengandungi keupayaan pelindung terhadap pembekuan.

Kadar pematangan oosit yang tidak matang sangat terjejas dengan pendedahan larutan vitrifikasi (39.6%) dan prosedur nyahvitrifikasi (33.9%). Kadar pematangan di antara oosit terdedah larutan vitrifikasi dan vitrifikasi tidak menunjukkan perbezaan. Keputusan ini menunjukkan kesan terjejas terhadap pematangan adalah berpunca dari larutan vitrifikasi dan bukannya prosedur vitrifikasi.

Perubahan ultrastruktur berpunca dari pembekuan dan vitrifikasi disiasat menggunakan mikroskop elektron transmisi dalam usaha untuk memperoleh jawapan dan penerangan terhadap keupayaan hidup yang rendah. Ruang perivitellin yang besar dan sedikit mikrovilli adalah perubahan ultrastruktur yang lazim berpunca dari penyejukbekuan.

Sungguhpun keupayaan hidup amat terjejas, kebanyakan organel oosit yang disejukbeku berupaya mengekalkan morfologi mereka.

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I certify that an Examination Committee met on 27th January 2003 to conduct the final examination of Myint Thein on his Doctor of Philosophy thesis entitled "*In Vitro* Viability and Ultrastructural Changes of Cryopreserved Immature Bovine Oocytes" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

Myint Thein

Date: 27 January 2003

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AFP	Antifreeze protein
A I	Anaphase I (First anaphase)
ART	Assisted reproductive technology(ies)
ATP	Adenosine triphosphate
BG	1,3-butylene glycol
BOEC	Bovine oviduct epithelial cell
BSA	Bovine serum albumin
CG	Cortical granule
CL	Corpus luteum
COCs	Cumulus-oocyte-complexes
conc	Concentration
CPA	Cryoprotective Agent
CS	Calf serum
DEG	Diethylene glycol
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
D-PBS	Dulbecco's phosphate-buffered saline
dpi	Day(s) post-insemination
EG	Ethylene Glycol
EGF	Epidermal growth factor
EME	Ethylene glycol monomethyl ether